REMARKS

Claims 1-40 are canceled. Claim 70 is added. Claims 41, 44-46, 50-52, 55, 57 and 64 are amended. Hence, claims 41-70 are now active and under consideration in this case.

At the outset, Applicants wish to gratefully acknowledge the previous rejections, now withdrawn, as set forth at pages 2 and 3 of the Official Action of December 21, 2011.

Before setting forth the bases for the patentability of the claimed invention over the cited references of record, attention is directed to the following in new claim 70, which replaces previous claim 40:

1) New claim 70 addresses the claim objections and rejections set forth by the examiner under 35 USC 112, second paragraph, in paragraphs 4 and 6 of the Official Action.

2) New claim 70 specifies that the restriction enzyme E1 is a type II restriction enzyme and the restriction enzyme E2 is a type IIS restriction enzyme. Support for these amendments may be found throughout the present specification and in Figures 2, 4-1, 4-2, 5-1, 5-2, 6-1 and 6-2, which show that: (i) the restriction enzyme E1 is a type II restriction enzyme because it recognizes and cleaves DNA at the same site (the recognition site is the restriction site, as shown in figures 4-1, 5-1 and 6-1), (ii) the restriction enzyme E2 is a type IIS restriction enzyme because it recognizes DNA at a site which is different and distant from the cleavage site; figures 4-2, 5-2 and 6-2 show that the cleavage site of the restriction enzyme E2 is downstream of its recognition site, as specified in claim 70.

3) In Claim 70, steps b) and c) have been clarified to better define the claimed invention;

4) In claim 70, the typographical error in step a) and the improper speacing in step b) have been addressed.

5) All of the amendments to claim 70 are fully supported by the claims and specification as originally filed, and, hence, no new matter has been added.

PRIOR ART REJECTIONS

Claims 40-43 and 45-69 stand rejected under 35 USC 102(b) as being anticipated by **Sapolsky et al** (US 2003/0008292, January 2003).

Claims 40-43 and 45-69 stand rejected under 35 USC 102(b) as being anticipated by **Yu et al** (WO 0234939, May 2002).

Claims 40-43 and 45-69 stand rejected under 35 USC 102(b) as being anticipated by **Van Eijk et al** (WO 0149882, July 2001).

Claims 40-43 and 45-69 stand rejected under 35 USC 102(b) as being anticipated by **Kato** (EP 0735144, October 1996).

Claim 44 stands rejected under 35 USC 103(a) as being unpatentable over any one of the above four cited references in view of **Keith et al** (US 5,093,245).

However, none of the five cited references would have anticipated or rendered the claimed invention obvious to one skilled in the art at the time the claimed invention was made.

Present claim 70 provides for the selective fragmentation of DNA as follows:

1) In step a), a type II restriction enzyme (E1) is used to generate DNA fragments (F1) with known terminal sequences (see Figures 4-1, 5-1 and 6-1);

2) In step b), ligation of the 5'-end of the DNA fragments F1 to the 3'-end of a double-stranded adapter AA' is effected to generate DNA fragments F'1. This step (b) specifies that the DNA fragments F'1 contain a sequence at the junction of the 3'-end of the adapter and the 5'-end of the DNA fragment (junction sequence) which consists of the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme E2. Therefore, the adapter AA' does not contain the entire sequence of the recognition site of a type IIS restriction enzyme, but only the 5'-terminal one or more base pairs of the sequence; and

3) Step c) uses the type IIS restriction enzyme (E2) to cleave a fraction of DNA fragments F'1 at their 5'-end to generate short DNA fragments. As noted above, step b) specifies that the junction sequence (i) consists of te 5'-terminal one or more base pairs but not the entire sequence of the recognition site of a type IIS restriction enzyme E2, and (ii) contains at its 3'-end the 3'-terminal one or more base pairs of the E1 restriction enzyme restriction site. Therefore, step b) means that the 3'-terminal base pairs of the E1 restriction site and the 5'-terminal base pairs of the E2 recognition site overlap over at least one base pair (see Figures 2, 4-1, 5-1, 6-1 and 7). This feature allows the selection of a fraction of short fragments F2 (step c) by cleavage with the restriction enzyme E2. These short DNA fragments F2 are derived from the fraction of the DNA fragments F'1 obtained in step b) which contains the entire recognition of the E2 enzyme (page 11, lines 1-13 and Figures 4-2, 5-2 and 6-2). The fraction of DNA fragments F'1 which are cleaved by the restriction enzyme E2 in step c) are formed by the adapter AA' and the DNA fragments F1 whose sequence contains at its 5'-end, the sequence of the 3'-terminal one or more base pairs of the recognition site of said restriction enzyme E2, which 3'-termianl base pairs together with the 5'-terminal base pairs from the junction sequence can form the entire recognition site of the restriction enzyme E2.

In summary, the claimed invention allows one to analyze a selective fragmentation

of nucleic acids by using a combination of:

i) A type II restriction enzyme (E1) to generate DNA fragments (F1) with known 5'-end and 3'-end sequences;

ii) A type IIS restriction enzyme (E2) which cleaves DNA downstream of its recognition site, such that the 3'-terminal base pairs of the E1 restriction site and the 5'-terminal base pairs of the E2 recognition site overlap over at least one base pair (see Figures 2 and 7 and page 11, lines 1-13 of the present specification); and

iii) A double-stranded adapter AA' which contains the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the enzyme E2.

As noted above, and as illustrated in Figures 1-8 of the present specification, only a fraction of the DNA fragments which contain the entire recognition site of the type IIS restriction enzyme E2 are cleaved, allowing the selective fragmentation of the DNA. The DNA fragments (F'1) which are cleaved by the restriction enzyme E2 are derived from the DNA fragments (F1) whose sequence contains at its 5'-end, the sequence of the 3'-terminal one or more base pairs together with the 5'-terminal base pairs from the junction sequence can form the entire recognition site of the restriction enzyme E2.

The short DNA fragments (F2) generated by the claimed method are unique in that the specific sequence (i.e., the sequence from the cDNA or genomic DNA to be analyzed) is flanked by the recognition and the cleavage site of the E2 enzyme and the 5'-end of the specific sequence is identical to the last base pairs of the E2 restriction enzyme recognition site. (See Figures 4-2, 5-2 and 6-2)

At page 13 of the current official Action, the examiner asserts that neither the claims nor the specification provide a definition of an E1 or E2 enzyme restriction site. However, this is not true. As already noted above, claim 70 now recites a combination of a type II restriction enzyme (E1) and a type IIS restriction enzyme (E2) whose

cleavage site is downstream of the recognition site of the E2 enzyme, wherein the 3'-terminal base pairs of the E1 restriction site and the 5'-terminal base pairs of the E2 recognition site overlap at least one base pair. See Figures 2, 4-1, 5-1, 6-1 and 7. Thus, the claimed method clearly provides a definition for an E1 or E2 enzyme restriction site.

At page 13 of the current Official Action, the examiner asserts that the claims would inherently encompass fragments having type IIS RE recognition site sequence. However, this is not true. As mentioned above, the short DNA fragments (F2) generated by the claimed method are unique in that the specific sequence (i.e., the sequence from the cDNA or genomic DNA to be analyzed) is flanked by the recognition and the cleavage site of the E2 enzyme and the 5'-end of the specific sequence is identical to the last base pairs of the E2 restriction enzyme restriction site. See Figures 4-2, 5-2 and 6-2.

None of the cited references, either alone or in combination, discloses or suggests either the claimed method for generating short DNA fragments or the short DNA fragments obtained by the claimed method.

Sapolsky et al (US 2003/0008292) merely discloses (see Figures 2 and 3, and pages 2-9 thereof) a method for identifying sequence based nucleic acid markers containing (paragraph 34, page 3):

1. Digesting nucleic acids with a first type IIS restriction enzyme (RE) having a recognition site on the nucleic acids sequence (*Ear*I site: 5'-CTCTTC3'), thereby cleaving the sequence dowmstream of its recognition site;

2. Ligating to the cleaved sequence a first adapter which contains a second type IIS RE recognition site; the adapter contains the complete sequence of the type IIS RE recognition site (*Hga*II site: 5'-GCGTC3') as shown in Figures 2 and 3;

3. Digesting the ligated sequence with the second type IIS RE that cleaves upstream of its recognition site and within the first type IIS RE recognition site (see Figures 2 and 3), thereby cleaving the sequence; and

4. Ligating the cleaved sequence with a second adapter sequence, thereby generating short DNA fragments having an ambiguous sequence (specific sequence from the nucleic acid sample) sandwiched between two different type IIS RE recognition sites (5'-CTCTTCNnnnGCGTC3'); Figures 2 and 3 and page 3, middle of paragraph 34.

Sapolsky et al describe a method for preparing short DNA fragments using: (i) two Type IIS restriction enzymes (RE), a first type IIS RE cleaving DNA upstream of its recognition site and a second type IIS RE cleaving DNA upstream of its recognition site, and (ii) a double-stranded adapter containing the entire sequence of the second type IIS RE.

Thus, **Sapolsky et al** fails to either disclose or suggest the claimed method for preparing short DNA fragments using: (i) a type II (E1) and a type IIS (E2) RE such that the E1 restriction site 3'-terminal sequence and the E2 recognition sire 5'-terminal sequence overlap at least one base pair, and (ii) a double-stranded adapter containing the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme (E2).

Also, the short DNA fragments generated with the method of Sapolsky et al contain a specific sequence flanked by two different type IIS RE recognition sites.

Clearly then, **Sapolsky et al** would have failed to either disclose or suggest the claimed invention to one skilled in the art at the time the claimed invention was made.

Yu et al merely disclose (see pages 5,6 and 11-15 and Figure 2) a method of analyzing DNA, entailing:

1. Ligating a first oligonucleotide (adapter, Figure 2) and a DNA fragment (obtained by digestion with a RE including a type II RE) to form a ligated product. The adapter contains a known sequence and a type IIS RE recognition site, i.e., the complete sequence of the type IIS RE recognition site (*Gsu*I site: 5'-CTGGAG3') as shown in Figure 1a; and

2. Digesting the ligated fragments with the type IIS RE to release a second nucleotide containing the first oligonucleotide and a sequence from the DNA fragment.

Yu et al disclose a method for preparing short DNA fragments using: (i) a type II and atype IIS RE and (ii) a double-stranded adapter containing the entire sequence of the type IIS restriction enzyme (E2).

Notably, while Yu et al generates short DNA fragments having a specific sequence (sequence from the DNA fragment) flanked by the recognition and the cleavage site of a type IIS RE, the 5'-end of the specific sequence does not contain the 3'-end sequence of the tpe IIS RE recognition site because the complete sequence of the type IIS recognition site is in the adapter. See Figure 2.

Hence, this reference would have failed to either disclose or suggest the claimed invention to one skilled in the art at the time the claimed invention was made.

Van Eijk et al merely disclose (see pages 1-10, 24-25 and 27-28 and Figure 1) amethod for generating an oligonucleotide (short DNA fragment) entailing:

1. Providing a first dsDNA;

2. Ligating the first dsDNA to a second dsDNA containing a type IIS recognition site (i.e., the complete sequence of the type IIS RE recognition site), so as to provide a ligated dsDNA; and

3. Restricting the ligated DNA with the type IIS RE so as to obtain a first and a second IIS-restricted dsDNA.

Importantly, this reference, thus, fails to either disclose or suggest the use of: (i) the E1 restriction site 3'-end sequence and the E2 recognition site 5'-end sequence overlapping over at least one base pair, and (ii) a double-stranded adapter containing the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme (E2). Without these elements, one skilled in the art could not have been put in possession of the claimed invention.

Kato merely discloses (pages 5 and 6 and 11-13, Figures 11-13) a method for molecular indexing of expressed genes using a combination of type IIS restriction enzymes, which entails:

1. Digesting nucleic acids (cDNA) with a first type IIS restriction enzyme (RE);

2. Ligating each of the resultant cDNA fragments to one from a pool of 64 biotinylated adaptors cohesive to all possible overhangs; and

3. Digesting the resultant cDNA fragments further with a second type IIS RE which is different from the first type IIS RE, thereby obtaining a first cDNA sample.

Thus, **Kato** discloses a method for preparing short DNA fragments using: (i) two type IIS RE and (ii) a double-stranded adapter complementary to one of the NNNN cohesive ends generated by the first type IIS RE. Kato does not use adapters containing type II recognition site. The type IIS recognition sites are in the DNA sequence. See Figures 1 and 3.

Clearly, **Kato** fails to disclose or suggest the claimed method for preparing short DNA fragments: (i) using a type II (E1) and a type IIS (E2) RE such that the E1 restriction site 3'-end sequence and the E2 recognition site 5'-end sequence overlap over at least one base pair, and (ii) a double –stranded adapter containing the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme (E2). Rather, to generate a subset of short DNA fragments from an initial set of DNA fragments, **Kato** discloses ligating the ends of the DNA fragments generated by a type IIS RE to a double-stranded adapter complementary to one of the NNNN cohesive end generated by the first type IIS RE (Figures 1 and 3). Thus, one skilled in the art would have been motivated from this reference to use selective ligation, i.e., ths, quite unlike the claimed invention.

Also, the method of **Kato** generates short DNA fragments containing a specific sequence flanked by two type IIS cleavage sites (see Figure 1; step(4) fragment (i)).

Clearly, this reference would have failed to put one skilled in the art in possession of the claimed invention at the time it was made.

Furthermore, it is equally clear that none of the references cited under 35 USC 102(b), even in combination, would have disclosed or suggested the claimed invention to one skilled in the art at the time it was made.

Hence, all four grounds of rejection under 35 USC 102(b) are believed to be unsustainable and should be withdrawn.

Likewise, the ground of rejection under 35 USC 103(a) is also unsustainable.

Keith et al is cited merely as disclosing a method of simultaneously preparing DNA fragments by restriction enzyme digest and ligating the fragments to another nucleic acid of desired function.

However, this reference also fails to either disclose or suggest the use of: (i) using a type II (E1) and a type IIS (E2) RE such that the E1 restriction site 3'-end sequence and the E2 recognition site 5'-end sequence overlap over at least one base pair, and (ii) a double-stranded adapter containing the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme (E2).

Clearly, even the combined disclosures of all five (5) cited references would have failed to provide motivation, guidance or enablement to one skilled in the art to attain the claimed invention. Necessarily then, one skilled in the art would have had no reasonable expectation of success in practicing the claimed invention as there is not even implicit motivation in the cited prior art to make and use the claimed invention. *KSR v. Teleflex*, 550 US 398 (2007). Furthermore, the internal memorandum of Margaret Focarino, Deputy Commissioner of Operations, USPTO, has made it clear-in the aftermath of *KSR*- that U.S. patent examiners still have the burden "to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed." Even if one skilled had some motivation to combine all of the cited references, the artisan still would not have arrived at the claimed invention.

Thus, in view of all of the above, all prior art bases of rejections are deemed to be unsustainable and should be withdrawn.

REJECTION UNDER 35 USC 112, second paragraph

Claims 40-56 and 60-69 stand rejected under 35 USC 112, second paragraph as ostensibly being vague and indefinite.

However, it is perfectly clear from new claim 70 that the junction sequence which results from the ligation of the 5'-end of the DNA fragments F1 to the double-stranded adapter AA' is located at the junction of the 3'-end of the adapter and the 5'-end of the DNA fragments F1, rendering the rejection moot.

Furher, it is now quite clear from claim 70, step a) that the DNA fragments which are generated by one or more type II restriction enzymes which generate blunt or cohesive ends. Both ends of the DNA fragment can be blunt or cohesive, or one end can be blunt and the other cohesive, depending upon the type of enzymes used (i.e., those generating one or the other or both ends) and their frequency of cleavage (see claims 41-43 and Figure 8).

It is also quite clear from step b) of claim 70 that the 5'-end of the DNA fragments is ligated to the adapter AA'. The 3'-end of the DNA fragment is also ligated to the adapter AA' when step a) is performed with one restriction enzyme E1 only.
Alternatively, the 3'-end is ligated to the adapter CC' when two restriction enzymes ($E1_A$ and $E1_C$) are used in step a). See claims 41-43 and Figure 8. However, the 3'-end of the DNA fragment is not important for the claimed method because short DNA fragments containing a portion of genomic or cDNA sequence can be generated by

cleavage at the 5'-end but not at the 3'-end because the restriction enzyme E2 cleaves the DNA downstream of its recognition site.

Furthermore, it is well-known in the art that adapters can be ligated to a blunt end or a protrusive end of a DNA fragment. Thus, the adapter AA' is ligated its 3'-end to the 5'-end of the DNA fragments (F1) which may be either blunt or cohesive, as described above.

The structure of the junction sequence is well-defined in claim 70 because step b) specifies that "the junction sequence consists of the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of a type IIS restriction enzyme E2, the cleavage site of which is located downstream of said recognition site, and wherein said junction sequence includes at its 3'-end the terminal one or more base pairs of the E1 restriction enzyme restriction site.

The fact that the junction sequence does not contain the entire sequence of the recognition site of the restriction enzyme E2 is not in contradiction with step c) because step c) specifies that it is only a fraction of the DNA F'1 which is cleaved in step c). As explained above, the fraction of DNA fragments F'1 which are cleaved by the restriction enzyme E2 are formed of the adapter AA' and the DNA fragments F1 whose sequence contains at its 5'-end, the sequence of the 3'-terminal one or more base pairs of the recognition site of the restriction enzyme E2, which 3'-terminal base pairs together with the 5'-terminal base pairs from the junction sequence can form the entire recognition site of the restriction enzyme E2 are thus cleaved by the E2 restriction enzyme in step c). By contrast, the DNA fragments F'1 which are formed of the adapter AA' and DNA fragments F1 whose sequence does not contain at its 5'-end, the sequence of the 3'-terminal one or more base pairs of the recognition site of the restriction enzyme E2 cannot be cleaved by the restriction enzyme E2 in step c) because they do not contain the entire sequence of the recognition site of the restriction enzyme E2.

In fact, the structure of the junction sequence is exemplified in Figures 4 to 6 and the detailed description of the Figures at pages 23-27 of the present specification.

In Figure 4-1, E1 is *Eco*RI having the recognition site 5'-G*AATTC-3' (cleavage site Indicated by the * on the sense strand) which generates DNA fragments F1 having cohesive ends with AATT and E2 is *Mme*I having the recognition site (5'-TCCPuAC-3'); The cleavage site of *Mme*I is distant from the recognition site (20 bases downstream of Pu on the sense starnd, as indicated in Figure 2).

The DNA fragment F'1 is formed by ligation of the 5'-end of the DNA fragments F1 to the adapter AA' which is complementary to the AATT ends generated by *Eco*RI, has the following sequence at its 5'-end:

5'-GGAAGCCTAGCTGGAC**AATTCN$_1$N$_2$N$_3$N$_4$N$_5$NNNNNNNNNN-3'**

3'-CCTTCGCATCGACGTCTTA<u>A</u>**GN$_1$N$_2$N$_3$N$_4$N$_5$NNNNNNNNNN-5'**

Adapter AA' junction Sequence DNA fragment F1

The adapter AA' contains only the first nucleotide (A on the antisense strand) of the *Mme*I recognition site.

The junction sequence is TC (on the sense strand) which consists of the two 3'-terminal bases of *Eco*RI recognition site and the two 5'-terminal of the *Mme* I recognition site on the sense strand.

Only the DNA fragments F'1 having CPuAC in $N_1N_2N_3N_4$ of the sense strand will form the entire recognition site of E2. This subset of DNA fragments F'1 will be selectively cleaved by E2 to obtain a fraction of short DNA fragments F2 (Figure 4-2).

Accordingly, in Figure 5-1, the junction sequence is TCC (on the sense strand) which consists of the three 3'-terminal bases of the *BamH*I recognition site and the three 5'-terminal of the *Mme* I recognition site on the sense strand. The adapter AA' contains only the first two nucleotides (AG on the antisense strand) of the *Mme*I recognition site. Only the DNA fragments PuAC in $\mathbf{N_1N_2N_3}$ of the sense strand will form the entire recognition site of E2. This subset of DNA fragments F'1 will be selectively cleaved by E2 to obtain a fraction of short DNA fragments F2 (Figure 5-1).

In Figure 6-1, the junction sequence is GGCGG (on the sense strand) which consists of the four 3'-terminal bases of the *Ksp*I recognition site and the four 5'-terminal of the *Eci*I recognition site on the sense strand. The adapter AA' contains only the first three nucleotides (GGC on the sense strand) of the *Eci*I recognition site. Only the DNA fragments F'1 having A in $\mathbf{N_1}$ of the sense strand will form the entire recognition site of E2. This subset of DNA fragments F'1 will be selectively cleaved by E2 to obtain a fraction of short DNA fragments F2 (see Figure 6-2).

Clearly, in view of the above remarks, this ground of rejection is deemed moot.

REJECTION BASED ON NON-STATUTORY DOUBLE PATENTING

Claims 40-69 stand rejected under the grounds of non-statutory double patenting as being unpatentable over claims 1-14 and 25 of copending USSN 12/458,610.

However, in view of what has been said above regarding the claimed method and what it entails, none of claims 1-14 or 25 of USSN 12/458,610, recite:

1) Step a) performed with a type II restriction enzyme (E1);

2) Step b) performed with a type IIS restriction enzyme (E2) such that the

3'-terminal base pairs of the E1 recognition site and the 5'-terminal base pairs of the E2 recognition site overlap over at least one base pair, and

3) The adapter AA' contains the 5'-terminal one or more base pairs but not the entire sequence of the recognition site of the type IIS restriction enzyme.

Quite clearly, the present claims are much more specific than the generic claims of USSN 12/458,610. Additionally, the present claims are patentably distinct over claims 1-14 and 25 of the cited co-pending application.

Hence, this ground of rejection is deemed moot.

Accordingly, in view of all of the above, it is believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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